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THE TANNIC ACID METHOD FOR QUANTITATIVE DETERMINATION OF CARBON MONOXIDE IN THE BLOOD.[1]

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INTRODUCTION.

Carbon monoxide may occur in many places, and inhalation of that insidious gas is a frequent and widely distributed cause of poisoning, ranging in severity from headache and inefficiency to unconsciousness and death. People are continually being affected by carbon monoxide in the home, in garages, around gas and gasoline engines and blast furnaces, in fighting fires, after blasting in mines and quarries, and after mine fires and explosions; in fact, everywhere there is the possibility of an exposure to the products of combustion of carbonaceous fuels or products. On the other hand, there are many cases reported where ill effects and accidents are wrongly attributed to carbon monoxide. Therefore it is essential for doctors, coroners, safety engineers, and first-aid men to be able to recognize this poisoning, not only in order to give proper treatment and to determine the cause of death, but also to insure just decisions on claims and to eliminate illusions and complaints of workmen.

As the ordinary symptoms of carbon monoxide poisoning, such as headache, nausea, dizziness, collapse, and unconsciousness, can be caused by other things, and may also vary with the individual, there is no specific test for the degree of poisoning except an examination of the blood for carbon monoxide-hemoglobin (CO–Hb) content. To make this test from a purely qualitative consideration is comparatively easy, and a number of procedures have been devised. However, in view of the fact that the occurrence of symptoms is in proportion to the degree of poisoning, it is desirable not only to detect the presence of the CO–Hb, but also to determine the quantity. Heretofore the methods used to make this quantitative estimation have either required elaborate and expensive apparatus, or involved technique unsuited for conditions except those found in the laboratory. Owing to the fact that most of the cases of poisoning occur at places distant from laboratories making these examinations, and also to the obvious necessity of immediate results, there is a demand for a reliable method which can be used by any person likely to have contact with cases of carbon monoxide poisoning. A suitable method is outlined below.

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[1] Investigations carried on in cooperation with the United States Bureau of Mines.

DEVELOPMENT.

Following the procedure of Wetzel's[2] qualitative test, when normal blood, diluted 1:4 with water, and shaken with an equal volume of 1 per cent tannic acid, is allowed to stand for 24 hours, a gray suspension is formed, while blood containing CO-Hb remains a carmine red. This color reaction is quite sensitive and, when once formed, changes so slowly that the writers decided to adapt it to quantitative purposes.

From two specimens of blood—the one being ordinary venous blood and the other blood saturated with CO—solutions were made with distilled water (1:9), and using these, mixtures were prepared varying from none to 100 per cent CO-Hb (0, 10, 20, etc.). Equal volumes of these mixtures were then put in each of a series of test tubes and treated with tannic acid. When arranged in a rack and left standing for 10 hours the graduation of a color change with the amount of CO-Hb was quite evident. A sample of an "unknown" blood, prepared and conditioned similarly, could be readily matched with its corresponding standard.

The results of the preliminary work promised an accurate yet easy method for quantitative determination of CO in the blood, and it was decided to investigate it further in view of finding the best working conditions—that is, concentration of blood and acid, size of test tubes, and proper lighting conditions for making the comparison of unknowns to the standards. Later, the effects caused by the use of anticoagulants[3] in preserving the blood to be used as material for standards, the storage of blood for standard material, the possibility of decreasing the time required for the formation of the color, and the permanency of the prepared standards were also investigated.

PROCEDURE FOR MAKING STANDARDS.

As the result of a rather extensive series of tests on the above conditions, the procedure finally adopted for making standards is as follows:

Using a modified Keidel tube (work cited) or any intravenous means, 5 c. c. or more of human blood is drawn and kept from clotting by the addition of 0.05 gram of potassium citrate ($K_3C_6H_5O_7$), or 0.02 gram sodium fluoride (NaF) (work cited) per 10 c. c. of whole blood (the advantages of each will be discussed later). The blood thus obtained is divided into approximately equal parts, one of which is immediately diluted 1 in 10 with distilled water, while the other is saturated with 3 to 5 per cent of CO gas, then diluted 1 in 10 with distilled water. The saturating of blood with CO should be done before diluting with distilled water, so as to minimize the volume of CO gas dissolved in the solution, because the physical solubility is thus limited to the plasma; whereas, if the saturation were made after dilution with water the volume of gas dissolved would have to be so increased, in order to insure saturation of the hemoglobin in the diluted solution of venous blood, that it would affect seriously the calculated relation.

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[2] McNally, W. D.: Carbon monoxide poisoning. Jour. Am. Med. Assn., Nov. 10, 1917.

[3] O'Brien, H. R., Jones, C. W., Allison, V. C.: Collection and preservation of blood samples for carbon monoxide determination. (Unpublished.) United States Bureau of Mines.

From these solutions of approximately all oxy-hemoglobin (O_2Hb) and carbon monoxide-hemoglobin (CO–Hb), respectively, mixtures are made which total 1 c. c., but vary from none to 100 per cent CO–Hb in steps of 10. These are contained in test tubes of approximately $\frac{5}{16}$ inch inside diameter, and of clear, thin, glass. To each standard thus prepared is added 1 c. c. of a mixture consisting of equal parts of a strictly fresh solution of 2 per cent pyrogallic acid and a solution of 2 per cent tannic acid, after which the tube is inverted twice to insure thorough mixing. Immediately after adding the acid, the tube should be sealed by pouring a little melted paraffin on top of the contents, the tube being immersed in cold water as a caution against overheating. This temporary seal will be sufficient to exclude the air until the walls of the tubes have become dry, after which a tight, permanent seal can be made by placing a disk of cardboard on top of the paraffin and filling the remainder of the tube with ordinary sealing wax. Care should be taken to have no air inclosed between the disk and the parafin, or between the disk and the sealing wax. Standards thus prepared develop their full color in from 10 to 15 minutes, and if properly sealed will remain in a suitable condition of permanency for several weeks.

TECHNIQUE OF DETERMINATION.

In making an estimation of the CO in the blood of a supposed victim of poisoning, the technique is, in principle, quite similar to that of making the standards. Measure into a test tube of the same size and glass as used for the standards, 1 c. c of a 0.05 per cent solution of potassium citrate or 0.03 per cent solution of sodium fluoride, depending on the anticoagulant used in preparing the standards. Then, by the aid of a hemospast, make a small puncture wound in the tip of the sterilized finger of the subject, and, with a capillary pipette, draw up 0.1 c. c. of blood. Quickly discharge this into the solution in the test tube, and add 1 c. c. of the mixture of pyrogallic and tannic acids as previously described. After inverting twice to mix the constituents thoroughly, and allowing to stand from 8 to 10 minutes, a comparison with the standards can be made and the percentage noted. It is very good practice to prepare a similar sample from an unexposed subject, thus insuring against spoiled reagents or change in standards. Any differences noted in this control should be considered in estimating other samples.

The comparison of unknowns with standards can be made in various ways. Our procedure is to hold the unknown in the hand together with a row of four or five standards, and move its position until it, by color comparison, fits between two of them, after which an estimation can be made on the basis of the value of the two standards; or comparison can be made in a test-tube rack having fixed positions for the standard and a space between each for the interposition of the unknowns. In the use of this latter, the unknown is again moved until a place is found where it is, say, a little more red than the 20 and a little less than the 30.

In making the comparison, the observer should stand with his back to the light, viewing the tubes by reflection, and should change the position of the tubes several times to note if any differences occur that are due merely to unequal lighting effect. Also, holding the tubes against a black background, such as a coat sleeve, seems to facilitate the observation.

ACCURACY OF METHODS.

The color graduation is so marked that the untrained observer can obtain sufficiently dependable results. At various times during some investigations on the effects of CO poisoning, untrained observers were asked to estimate unknowns at the same time as the writers; also the results obtained were in many instances compared with other methods. The following tables give typical analysis:

TABLE I.—*Readings of five observers.*

Per cent saturation as made up.	Per cent as read by observers.				
	1	2	3	4	5
75	80	70	70	75	75
65	60	60	60	60	60
38	35	40	40	35	40
15	20	10	15	20	15
5	5	5	10	10	5

TABLE II.—*Readings of three observers and variations from actual percentage.*

Per cent CO as made up.	Per cent read by observers.					
	1		2		3	
	Reading.	Variation.	Reading.	Variation.	Reading.	Variation.
0	0	0	0	0	1	1
5	10	5	10	5	10	5
15	15	0	15	0	15	0
28	26	2	30	2	25	3
44	44	0	44	0	45	1
85	85	0	85	0	88	3

TABLE III.—*Comparison of tannic acid with spectrophotometric method.*

Time after leaving gas chamber (minutes).	Per cent saturation with CO.		Time after leaving gas chamber (minutes).	Per cent saturation with CO.	
	Spectrophotometer.	Tannic acid.		Spectrophotometer.	Tannic acid.
2	35.0	36.0	48	12.0	19.0
14	31.0	28.0	123	9.2	12.0
30	22.6	24.0	314	5.0	6.0

These results given in Table III were obtained after the subject (Sayers) had been exposed for 55 minutes to 16 parts of CO in 10,000 parts of air. It will be noted in the comparison of the two methods that with but one exception the variation is less than 3 per cent, which is negligible for practical work in the determination of CO in the blood.

TABLE IV.—*Comparison of the tannic acid with the spectrophotometric, carmine, and Van Slyke methods for the determination of CO in the blood.*

Method and observer.	Per cent saturation.	Average.	Method and observer.	Per cent saturation.	Average.
Spectrophotometer-1	70		Carmine-3	74	
Do	73	71.5	Do	76	75
Tannic acid-2	72	72	Van Slyke-4	70	70

In this experiment, a dog weighing 15 pounds had been exposed to 63 parts of CO in 10,000 for 30 minutes, and, after being treated with 90 per cent O_2 and 10 per cent CO_2, died. The blood was obtained from the heart by puncture.

TABLE V.—*Comparison of tannic acid method with spectrophotometer and results calculated from alveolar air.*

Tannic-acid method.	Spectrophotometer.	Calculated from alveolar air.	Tannic-acid method.	Spectrophotometer.	Calculated from alveolar air.
20	18	18	10	8	14
11	14	12	22	18	23
10	11	7	18	16	14
7	11	9			

TABLE VI.—*Comparison of tannic acid method with the Van Slyke method for the determination of CO in specimens of blood which had been preserved for 12 days with NaF.*

Tannic acid.	Van Slyke.	Tannic acid.	Van Slyke.
35	40	20 [1]	23
35	35	30	34

[1] Blood clotted and slightly decomposed.

These results were obtained routinely and by different observers. It is evident that an occasional analysis by one man might be incorrect by 5 per cent, but the error is generally less.

DISCUSSION AND PRECAUTIONS OF THE PROCEDURE.

In view of the simplicity of the procedure, and the reliability of the results, this method should be well suited to the determination of CO poisoning. It can also be used to estimate the CO–Hb in samples which have been collected in the field and shipped to the laboratory; and its dependability for research problems has been illustrated in connection with some recent work, data from which are shown in Tables III, IV, V, and VI.

When using this method for the estimation of samples that were collected in the field, it is of importance to know what anticoagulant was used, and to have the standards made from a like preserved stock, otherwise there will be a difference of color. For immediate routine work, potassium citrate is better than sodium fluoride because it produces a lighter color in which it is much easier to detect the carmine red. On the other hand, where three days or more will elapse between the taking of the sample and its analysis, it would be well to use sodium fluoride, owing to its better preserving properties, even though it produces a darkening effect which masks the color. However, this estimation of field samples is secondary to the main use of the method, and for nearly all ordinary work, potassium citrate will be quite suitable.

Probably the most important item of the entire analysis is to have strictly fresh prepared solutions, especially the mixture of tannic and pyrogallic acids. In order to insure this, the scheme best suited is to have ampules prepared containing the acids in the solid form. When needed for use these can be broken, and the contents emptied into water in which the acid quickly dissolves and is ready for use. The potassium citrate solution does not deteriorate as quickly as the acid mixture, but will spoil in time, and it might also be good practice to prepare it fresh from ampules.

In order to avoid having to obtain fresh blood for standards, we are preparing a permanent set from pigments, and this promises to furnish what is ultimately expected of the method, namely, a standard which can easily be kept in readiness for use at all times and without any preliminary preparation.

SUMMARY.

We believe that the method described is particularly adapted to the requirements for determination of poisoning by CO gas. It can be used for either a quantitative estimation or, in the absence of a set of standards, as a qualitative test, the comparison in the latter being made with a single standard immediately prepared from 0.1 c. c. of the blood of an unexposed subject (very conveniently the person making the test). As support for the foregoing statement, the following conclusions are offered:

1. The small quantity of blood needed can easily be obtained without objection on the part of the patient.
2. The solutions used are cheap, common, and easily made. The apparatus used is simple and inexpensive.
3. The actual time of making an analysis is not more than 3 minutes; and results can be obtained in 8 to 10 minutes.
4. The accuracy is well within the required limits.
5. No great skill or special training is necessary for securing good results.
6. The method automatically corrects for any dissociation of carbon monoxide-hemoglobin due to the dilution with water.
7. The results are more easily obtained, and are, as a whole, more dependable than with any other method tried.

REQUIREMENT THAT MILK SELLER SECURE PERMIT UPHELD.

An ordinance of St. Louis, Mo., requires that a permit from the board of public service be obtained by any person selling milk. In a prosecution[1] for violation of the ordinance, where the defendant was charged with conducting a dairy and selling milk therefrom without having a permit, the Supreme Court of Missouri held that the requirement necessitating a permit was a lawful one. In so deciding the court said:

> It thus appears that the very purpose of the permit was to protect the public. It indicated to the purchasers that the municipality had determined that the holder of the permit could be relied upon for a wholesome quality of the article sold. It is like the license from the State board of health to a doctor. Such license bespeaks the qualifications of the holder, and thus protects the general public. The permits or licenses authorized by this ordinance perform the same function. They say to the general buying public, You will be safe in buying from this man (the holder of the permit) because he can be relied upon to furnish a wholesome article. And, further, the issuance of permits is but another method of registration of milk sellers. The city, in exercise of its police power, had the authority to require milk sellers to take out such permits.

DEATHS DURING WEEK ENDED SEPTEMBER 23, 1922.

Summary of information received by telegraph from industrial insurance companies for week ended September 23, 1922, and corresponding week 1921. (From the Weekly Health Index, September 26, 1922, issued by the Bureau of the Census, Department of Commerce.)

	Week ended Sept. 23, 1922.	Corresponding week 1921.
Policies in force	50, 614, 343	47, 083, 403
Number of death claims	7, 908	7, 482
Death claims per 1,000 policies in force, annual rate	8. 1	8. 3

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[1] City of St. Louis *v.* Kellmann, 243 S. W. 134.